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Dothiorella viticola on *Populus cathayana* in China: a new record

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Abstract — We document the first report of the species *Dothiorella viticola* (teleomorph: *Spencermartinsia viticola*) from China. This fungus was isolated from the bark of *Populus cathayana* collected from Qianyang, Shaanxi Provinces. Morphological characteristics of the anamorph indicated that it was a *Dothiorella* species, and phylogenetic analysis based on rDNA-ITS data confirmed that the isolate should be included within *Spencermartinsia*. Conidiogenesis, observed in pycnidia obtained in culture, was holoblastic with conidiogenous cells proliferating at the same level giving rise to periclinal thickenings. Conidia were brown, thick-walled and septate with both ends rounded or with a truncate base.

Key words — *Botryosphaeriaceae*, internal transcribed spacer.

Introduction

Species of *Botryosphaeria* Ces. & De Not. are well known as pathogens, saprophytes, and endophytes on a wide range of woody angiosperm and gymnosperm hosts (Barr 1972, von Arx 1987, Denman et al. 2000). While the morphology of the teleomorphic states differs little among species, a wide range of morphologies is seen in the anamorphs and it is on this basis that species are distinguished. Anamorphs of *Botryosphaeria* species currently are placed in the genera *Fusicoccum* Corda, Diplodia Fr., *Lasiodiplodia* Ellis & Everh., and *Dothiorella* Sacc. (Crous & Palm 1999, Denman et al. 2000, Phillips et al. 2005). These four genera are clearly distinct based on morphology and their

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phylogenetic relationships, as shown by analysis of sequence data of the internal transcribed spacer (ITS) of the rRNA repeat (Phillips et al. 2005).

The genus *Dothiorella* was resurrected to accommodate anamorphs of *Botryosphaeria* species with conidia that become colored and septate at an early stage of development, even before they are released from the conidiogenous cell (Phillips et al. 2005). This contrasts with conidia of *Diplodia* species, which are hyaline and become dark and septate only some time after they are formed, normally after a period of aging once they are discharged from the pycnidia (Shoemaker 1964, Alves et al. 2004, Phillips et al. 2005). Another distinctive morphological feature of *Dothiorella* anamorphs is their brown, one-septate ascospores (Shoemaker 1964, Sivanesan 1984, Alves et al. 2004).

This paper reports the identification of *Dothiorella viticola* A.J.L. Phillips & J. Luque, isolated from *Populus cathayana* in China. *Dothiorella viticola* (teleomorph: *Botryosphaeria viticola*) was described and illustrated in Luque et al. (2006), from pruned canes of *Vitis vinifera* in NE Spain. Recently, the teleomorph has been recombined as *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips et al., the sole species in the new genus *Spencermartinsia* (Phillips et al. 2008).

Materials and methods

Isolates. Pieces of diseased tissue were plated on potato dextrose agar (PDA) after 2 min of surface sterilization in 70% ethanol. Sporulation was enhanced by culturing the isolates on 2% water agar bearing pieces of autoclaved poplar branch at 25°C with a 12/12 h photoperiod. Growth rates were determined on PDA plates incubated in the dark at 25°C.

DNA sequencing. Template DNA was extracted from fungal mycelium according to the method of Barnes et al. (2001), and primer pairs used for amplification and sequencing of the ITS region were ITS1-F (Gardes et al. 1993) and ITS4 (White et al. 1990). Amplification was completed with the following cycling parameters: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 10 min. The PCR products were sequenced by Organism Technology Co., Ltd., Shanghai, China. Accession Number in GenBank is FJ786401 for isolate QY02.

The ITS nucleotide sequences generated in this study were added to other sequences downloaded from GenBank (Table 1), with high similarity according to a BLAST search (National Center for Biotechnology Information's nucleotide blast program). Preliminary alignments were performed using CLUSTAL-X. The alignments were imported into BioEdit 5.0.9.1 and manually adjusted. Phylogenetic analysis of aligned DNA sequences was performed with PAUP version 4.0b10 for 32-bit Microsoft Windows (Swofford 2001). Heuristic searches were performed with 1000 random sequence additions. The clade stability was evaluated by 1000 bootstrap replications. Other measures for parsimony, including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively), were also calculated. *Cercospora apii* was used as the outgroup taxon.

Table 1. Sequences used in the phylogenetic analysis of *Spencermartinsia* and *Botryosphaeria*.

Species	GenBank code	Host	Reference
Spencermartinsia viticola	FJ786401	*Populus cathayana*	This paper
S. viticola	AY905555	*V. vinifera*	Luque et al. 2006
S. viticola	AY905556	*V. vinifera*	Luque et al. 2006
S. viticola	AY905557	*V. vinifera*	Luque et al. 2006
S. viticola	AY905558	*V. vinifera*	Luque et al. 2006
B. australis	AY343388	*V. vinifera*	Van et al. 2004
B. australis	AY343385	*V. vinifera*	Van et al. 2004
B. corticola	AY259100	*Quercus suber*	Alves et al. 2004
B. corticola	AY259089	*Q. suber*	Alves et al. 2004
B. dothidea	AY640253	*Populus nigra*	Phillips et al. 2005
B. dothidea	AY259092	*V. vinifera*	Alves et al. 2004
B. iberica	AY573202	*Quercus ilex*	Phillips et al. 2005
B. iberica	AY573214	*Q. ilex*	Phillips et al. 2005
B. iberica	AY573213	*Q. ilex*	Phillips et al. 2005
B. lutea	AY259091	*V. vinifera*	Alves et al. 2004
B. lutea	AY236946	*Malus ×domestica*	Slippers et al. 2004
B. sarmentorum	AY573206	*Malus pumila*	Phillips et al. 2005
B. sarmentorum	AY573212	*Ulmus* sp.	Phillips et al. 2005
B. stevensii	AY259093	*V. vinifera*	Alves et al. 2004
B. stevensii	AY236955	*Fraxinus excelsior*	Slippers et al. 2004
B. rhodina	AY236952	*Pinus* sp.	Slippers et al. 2004
B. rhodina	AY236951	*Vitex doniana*	Slippers et al. 2004
B. obtusa	AY259094	*V. vinifera*	Alves et al. 2004
B. obtusa	AY236954	*Ribes* sp.	Slippers et al. 2004
B. parva	AY236943	*P. nigra*	Slippers et al. 2004
B. parva	AY259098	*V. vinifera*	Alves et al. 2004
Cercospora apii	AY179949	Unknown	Mostert et al. 2003

Results

Isolate QY02 was obtained in China from diseased tissue of *P. cathayana*, where it forms dark-brown, wart-like protuberances on the surface of trunks and branches. The warts on trunks and branches damage the tree, reducing its growth and productivity. colonies on PDA showed aerial mycelium and were cottony, dark olive to grayish, darkening from the center of the colony after 3 d; colony fully darkened after 6–10 d. Colonies on PDA reached 40 mm radius after 3 d at 25°C. pycnidia were produced after 20–30 d on 2% water agar at 23°C under near UV black light (12/12 h photoperiod). conidoma pycnidial, separate or aggregated into botryose clusters up to 2 mm diam, individual pycnidia

FIG. 1 A–D. Conidiogenous cells and conidia of *Dothiorella viticola* (*Spencermartinsia viticola*) Isolate QY02. B–C. Conidiogenous cell with periclinal thickenings. D. Conidia dark-brown, one-septate. E. Colony on water agar containing pieces of autoclaved poplar wood after 30d. Bars A–C = 5 μm, D = 10 μm

spherical to globose, black, unilocular, 200–360 μm diam, thick-walled, walls exhibiting three layers: an outer layer of dark brown, thick-walled cells textura angularis, a median layer of dark brown thin-walled cells textura angularis, and an inner layer of thin-walled, hyaline cells. CONIDIOPHORES hyaline, cylindrical, branched. CONIDIOGENOUS CELLS discrete or integrated, cylindrical to broad lageniform, 8–14 × 3–7 μm, hyaline, smooth, holoblastic, indeterminate, proliferating at same level to form periclinal thickenings. CONIDIA brown, oblong to subcylindrical, septate, occasionally slightly constricted at septum, moderately thick-walled, externally smooth, internally finely verruculose, ends rounded, often with a truncate base, 16–26 × 7–12 μm.

A multiple alignment of the rDNA-ITS was generated with 27 sequences obtained from GenBank plus the sequence of isolate QY02 from *Populus cathayana*. Two major clades were resolved in a MP tree with 242 length (CI = 0.8023, RI = 0.9272, RC = 0.7931) (FIG. 2). One clade, with 90% bootstrap support, contained four species with *Diplodia* and *Lasiodiplodia* anamorphs. The other major clade (83% bootstrap) consisted of three subclades containing isolates with *Fusicoccum* and *Dothiorella* anamorphs. The *Dothiorella* species grouped in a well-supported subclade (74%). Isolate QY02 and the five

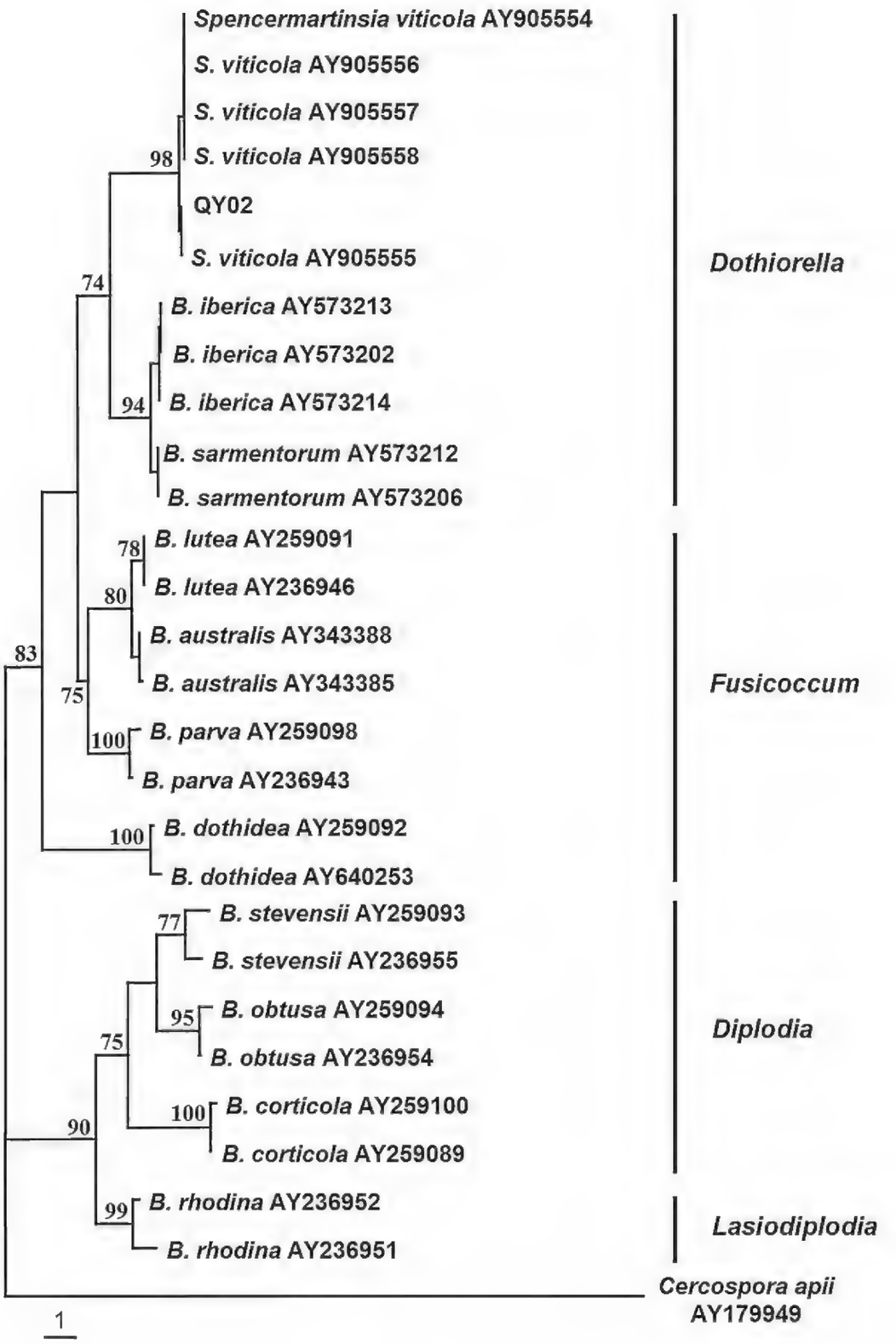

FIG. 2 The parsimony tree (TL = 242, CI = 0.8023, RI = 0.9272, RC = 0.7931) derived from a heuristic search option in PAUP version 4.0b10 with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 70% are indicated above or below the tree branches. The tree was rooted to *Cercospora apii*. Anamorph genera are indicated on the right.

S. viticola isolates from *Vitis vinifera* identified by Luque et al. (2006; as *B. viticola*) fell within a single clade with 98% bootstrap support.

Discussion

Based on phylogenetic analysis of the ITS region and morphological characters of the anamorph, we identified isolate QY02 as *Dothiorella viticola* (teleomorph: *Spencermartinsia viticola*). This study is the first report of *D. viticola* from poplar. This species was first described from grapevines (Luque et al. 2006) and previously known only from that host. The anamorph is morphologically distinct from *Diplodia* and can be accommodated in the genus *Dothiorella*. With *Botryosphaeria iberica* A.J.L. Phillips et al. and *B. sarmentorum* A.J.L. Phillips et al., *S. viticola* is the third species known to have an anamorph in *Dothiorella*. These three species are morphologically similar, but their differences are well supported by the ITS data (Luque et al. 2006). The teleomorph of *S. viticola* is reported to be "uncommon" on *Vitis vinifera* (Luque et al. 2006). Despite careful examination of diseased trunks and branches of *Populus cathayana*, we have not yet been able to find signs of the teleomorph of this fungus.

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